Chaperoning the synapse—NMNAT protects Bruchpilot from crashing

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Synapses perform several tasks independently from the cell body of the neuron, including synaptic vesicle recycling through endocytosis or local protein maturation and degradation. Failure to regulate protein function locally is detrimental to the nervous system as evidenced by neuronal dysfunctions that arise as a consequence of synaptic ageing. This relative synaptic autonomy comes with a need for mechanisms that ensure correct protein (re)folding, and there is accumulating evidence that key chaperones have a central role in the regulation and maintenance of synaptic structural integrity and function [1]. Work by Grace Zhai's group, published in this issue of *EMBO reports*, demonstrates a key role of the *Drosophila* nicotinamide mononucleotide adenyltransferase (NMNAT) chaperone in the protection of active zone components against activity-induced degeneration (Fig 1; [2]).

Active zones, the specialized sites for neurotransmitter release at presynaptic terminals, are characterized by a dense protein network called the cytomatrix at the active zone (CAZ). The protein machinery of the CAZ is responsible for efficient synaptic vesicle tethering, docking and fusion with the presynaptic membrane and, thus, for reliable signal transmission from the neuron to the postsynaptic cell. Clearly, proteins in the CAZ are tightly regulated, especially in response to external cues such as synaptic activity [3,4]. Yet, this particularly crowded protein environment might be favourable for the formation of non-functional—and sometimes toxic—protein aggregates. Chaperones that act at the synapse reduce the probability of crucial protein aggregation by preventing and reverting these inappropriate interactions, which happen as a result of environmental stress.

Fig 1 | Results reported by Zang and colleagues [2] reveal a specific role of nicotinamide mononucleotide adenyltransferase (NMNAT) in preserving active zone structure against use-dependent decline. This protection is exerted by direct interaction with BRP and protection of this key structural protein against ubiquitination and subsequent degradation. BRP, Bruchpilot; Ub, ubiquitin.

One of these chaperones, the *Drosophila* neuroprotective NMNAT, was identified in a genetic screen for factors involved in synapse function [5]. Its chaperone activity was later confirmed by using *in vitro* and *in vivo* protein folding assays [6]. NMNAT null mutants show severe and early onset neurodegeneration, whereas neurodevelopment does not seem to be strongly affected. Interestingly, degeneration of photoreceptors lacking NMNAT can be significantly attenuated by limiting synaptic activity, either by rearing flies in the dark or by introducing the no receptor potential A (*norpA*) mutation that blocks phototransduction [5]. These results indicate that NMNAT protects adult neurons from activity-induced degeneration.

In this issue of *EMBO reports*, Zang and colleagues report a role for NMNAT at the synapse. They observed that loss or reduced levels of NMNAT leads to a concomitant

loss of several synaptic markers including cysteine-string protein (CSP), synaptotagmin and the active zone structural protein Bruchpilot (BRP). Remarkably, BRP was the only one of these proteins found to co-immunoprecipitate with NMNAT from brain lysates. Both proteins show approximately 50% co-localization at the neuromuscular junction when imaged by 3D-SIM™ super-resolution microscopy, suggesting that NMNAT might act directly as a chaperone for maintaining a functional BRP conformation.

Consistent with a protective role of NMNAT against BRP degradation, RNA interference-mediated NMNAT knockdown leads to BRP ubiquitination, whereas this modification was not detected in control brain lysates. Given the involvement of the ubiquitin proteasome pathway in regulating synaptic development and function [1], the

authors tested the effect of the proteasome inhibitor MG-132 on BRP ubiquitination. They observed an increased level of BRP ubiquitination in wild-type flies fed with this drug, suggesting a role for the proteasome in the clearance of ubiquitinated BRP. By contrast, overexpression of NMNAT reduces the level of BRP ubiquitination both in the absence and the presence of MG-132, providing further evidence for the protective role of this chaperone against ubiquitination of BRP (Fig 1).

a key role of the [...] nicotinamide mononucleotide adenyltransferase (NMNAT) chaperone in the protection of active zone components against activity-induced degeneration

BRP is a cytoskeletal-like protein that is an integral component of T-bars—electron-dense structures that project from the presynaptic membrane and around which synaptic vesicles cluster. In agreement with a protective role of NMNAT against BRP ubiquitination, reduced levels of this chaperone give rise to a marked decrease in T-bar size in an age-dependent manner (Fig 1). Active zones are known to show dynamic changes in response to synaptic activity, and NMNAT was previously reported to protect photoreceptors against activity-induced degeneration [5]. The authors thus tested the effect of minimizing photoreceptor activity on active zone structure by keeping flies in the dark or inhibiting phototransduction by means of the *norpA* mutation. Both manipulations largely reversed the effect of NMNAT knockdown on T-bar size. Absence of light exposure also significantly reduced the amount of BRP that co-immunoprecipitates with NMNAT, indicating that neuronal activity regulates NMNAT–BRP interaction. Further experiments are needed to examine whether there is a positive correlation between synaptic activity and BRP

ubiquitination levels, and whether NMNAT can indeed keep T-bar structure intact by protecting BRP against this modification under conditions of high synaptic activity.

Finally, the study shows that reduced NMNAT levels not only caused a loss of BRP from the synapse but also a specific mislocalization of this protein to the cell body, where it accumulates in clusters together with the remaining NMNAT protein. Under these conditions BRP co-immunoprecipitated with the stress-induced Hsp70, a chaperone classically used as a marker for protein aggregation. It is still unclear whether these BRP clusters form as a result of defective anterograde trafficking and/or of enhanced retrograde transport of BRP. In the absence of light stimulation T-bars are properly assembled in *nmnat* null photoreceptors, but at this stage a role of NMNAT in regulating the axonal transport of BRP under conditions of normal synaptic activity cannot be excluded. Noticeably, two independent recent reports show involvement of NMNAT in mitochondrial mobility [7,8].

As BRP and NMNAT co-localize and interact with one another, the simplest model that accounts for all the observations by Zang *et al* is that NMNAT directly prevents activity-induced ubiquitination of BRP and subsequent degradation. Yet, as its name indicates, this chaperone is an essential enzyme in NAD synthesis. It was previously shown by the Bellen lab that mutant versions of NMNAT, impaired for NAD production, rescue photoreceptor degeneration caused by loss of NMNAT [5]. This strongly suggests that NAD production is not required for stabilization of BRP but this might need further scrutiny [9].

While providing further insights into the role of NMNAT at the active zone in *Drosophila*, the paper by Zang *et al* might also have important implications for neurodegeneration in mammals. When ectopically expressed in mice, *Nmnat* has a protective role against Wallerian degeneration, that is, synapse and axon degeneration that rapidly occurs distal from an axonal

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wound in wild-type animals. This process is significantly delayed in mice overexpressing a chimaeric protein consisting of the amino-terminal 70 residues of the ubiquitination factor E4B (Ube4b) fused through a linker to *Nmnat1*, known as the Wallerian degeneration slow (*Wld^s*) protein. Conversely, mutations in the human *NMNAT1* gene were characterized in several families with Leber congenital amaurosis—a severe, early-onset neurodegenerative disease of the retina [10–13]. As *Wld^s* or *Nmnat1* overexpression protects axons from degeneration in various disease models [9], *Nmnat1* emerges as a promising candidate for developing protective strategies against axonal degeneration in peripheral neuropathies such as amyotrophic lateral sclerosis but also in glaucoma, AIDS and other diseases [9].

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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